Reversal of Glomerular Lesions Involves Coordinated Restructuring of Glomerular Microvasculature

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Abstract. There is increasing evidence from both human and experimental studies that at least partial reversal of glomerulosclerosis can be achieved by glomerular remodeling. This requires substantial changes in glomerular architecture, specifically of glomerular capillaries. It was the purpose of the present study to characterize the stereologic and topologic characteristics of glomerular capillaries when partial reversal of glomerular lesions is achieved by high-dose angiotensin-converting enzyme inhibitor treatment. Sham-operated male Sprague-Dawley rats were compared with subtotally nephrectomized (SNX) rats. The latter were kept untreated for 8 wk and subsequently randomly divided into one group that was continued without treatment and another group that was treated with enalapril (48 mg/kg body wt per d administered in the drinking fluid for 4 wk). Renal morphology was evaluated after 8 or 12 wk, respectively, by stereologic techniques after pressure-controlled perfusion fixation. In SNX rats at 8 and particularly at 12 wk, the glomerulosclerosis index was significantly higher than in sham-operated rats. At 12 wk, it was lower in rats that had been treated for 4 wk with enalapril compared with untreated SNX rats, suggesting partial reversal of glomerular lesions. This was associated with a decrease in mean glomerular volume and mean glomerular tuft volume, a reduced number of capillaries per glomerulus, and reduced total length of capillaries per glomerulus but without any significant change in the length of individual capillaries. The numerical capillary density (Euler number density) as an index of topologic complexity did not change. The total capillary surface area per glomerulus was strikingly increased after subtotal nephrectomy and partially reversed after enalapril. This was accounted for primarily by fewer capillaries without any change in diameter. In parallel, the number of endothelial cells per glomerulus was strikingly increased after subtotal nephrectomy and decreased with enalapril treatment, but endothelial cell volume remained elevated. The study shows harmonious coordinate remodeling of the entire glomerulus during regression of glomerular lesions after subtotal nephrectomy. Proportionate reduction of glomerular volume and capillary number without change of individual capillary length were found. The numerical capillary density of the tuft therefore remained unchanged.

For a long time, the goal of therapeutic intervention in glomerular disease was to halt progression (1). More recently, it had become apparent that glomerular lesions in progressive renal diseases can be reversed to some extent presumably if there has been no major loss of podocytes. This concept was based on clinical observations of partial reversal of basal membrane thickening and mesangial expansion in diabetic patients several years after isolated pancreas transplantation (2). Furthermore, reversal of renal lesions was seen in patients with clinically cured IgA glomerulonephritis (3) as well as in experimental studies of Ikoma et al. (4), of our group (5), and of other laboratories (6,7). In the last studies, it had been shown that high-dose pharmacologic blockade of the renin angiotensin system causes partial reversal of glomerulosclerosis. Reversal of glomerulosclerosis, however, necessitates glomerular remodeling. This suggests major adjustment of glomerular, particularly capillary, architecture during the reversal process. The structural details of the remodeling process have not been clarified so far.

It has been shown that remodeling of the glomerulus recapitulates some of the processes, which are taking place during organogenesis and glomerular development in the fetal phase (8), but several questions had remained unresolved. Do glomerular volume and the capillary tuft volume change in parallel? Does capillary density remain constant? Is the reduction in capillary supply achieved by reducing the length or altering the geometry of individual capillaries? Does capillary sprouting occur during the complex restructuring of the microvascular architecture? For answering these questions, quantitative, unbiased, stereologic analysis, based on the fractionator/dis-
sector principle, was combined with topologic analysis according to Nyengaard et al. (9,10).

Materials and Methods

Animals

Male Sprague-Dawley rats (Charles River Co., Sulzfeld, Germany; mean body weight, 378 ± 15 g) were housed at constant room temperature (21°C) and humidity (75%) and exposed to a 12-h light-on/12-h light-off cycle. The animals had free access to water. The rats were fed pellets (Altromin, Lage/Lippe, Germany; 23.4% protein, 4.5% fat, 6% fiber, 0.40% sodium).

After a 7-d adaptation period, rats were randomly allotted to subtotal nephrectomy (n = 18) or sham operation (n = 6). As described before (11), the rats underwent two-step subtotal nephrectomy (removal of right kidney; 7 d later, weight-controlled surgical removal of cortical tissue of the hypertrophied left kidney corresponding to 66% of the weight of the right kidney). Eight weeks after the second operation, one group of rats was killed. The remaining animals were randomly allocated to two arms: no treatment or enalapril treatment (48 mg/kg body wt per d) for an additional 4 wk. The sham-operated animals were followed for 12 wk. Enalapril (Berlin Chemie, Berlin, Germany) was added to the drinking fluid at concentrations calculated to deliver the above dose. Food and water consumption were monitored daily, and the enalapril dose was adjusted accordingly.

The following four groups were studied: group 1, sham-operated rats that were killed 12 wk after sham operation (n = 6); group 2, subtotally nephrectomized (SNX) rats that were killed 8 wk after subtotal nephrectomy (n = 6); group 3, SNX rats that were killed 12 wk after subtotal nephrectomy (n = 6); and group 4, SNX rats that had delayed enalapril treatment (from 8 to 12 weeks after subtotal nephrectomy) and were killed 12 wk after subtotal nephrectomy (n = 6).

Tissue Preparation

Eight or 12 wk after surgery, blood samples were taken and the experiment was terminated by retrograde aortic perfusion with glutaraldehyde for morphometric and stereologic investigations. Perfusion pressure was kept constant at 120 mmHg. The adequacy of fixation had been examined in pilot experiments. The kidneys were weighed and dissected in a plane perpendicular to the interpolar axis, yielding slices of 1 mm width. Tissue slices were embedded in paraffin; 4-μm sections were prepared and stained with periodic acid-Schiff. Ten small pieces of one kidney were also selected by area-weighted sampling for embedding in Epon-Araldite. Five of the resin blocks were also randomly chosen, from which semithin sections (0.5 μm) were prepared and stained with methylene blue and basic fuchsin. Two of the resin blocks were also randomly chosen, from which 120 serial sections (1 μm) were prepared and stained. Subsequently, the kidneys were investigated by means of morphometry and stereology (9,10,11,12,13).

Morphologic Investigations

Glomerulosclerosis Index. All semiquantitative, morphometric, and stereologic investigations were performed in a blinded manner by an observer who was unaware of the study protocol. The degree of sclerosis within the glomerular tuft as an index of progression was determined on periodic acid–Schiff–stained paraffin sections adopting the semiquantitative scoring system proposed by El Nahas et al. (14). Using light microscopy at a magnification of ×400, the glomerular score of each animal was derived as the mean of 100 glomeruli. The severity of glomerulosclerosis was expressed on a scale from 0 to 4 (14). The glomerular score for individual glomeruli was as follows: grade 0, normal glomerulus; grade 1, beginning mesangial expansion/thickening of the basement membrane and/or irregular lumina of capillaries; grade 2, mild/moderate segmental hyalinosis/sclerosis involving <50% of the glomerular tuft; grade 3, diffuse glomerular hyalinosis/sclerosis involving >50% of the tuft; and grade 4, diffuse glomerulosclerosis with total tuft obliteration and collapse. The resulting index in each animal was expressed as a mean of all scores obtained.

Glomerular Geometry. Area (Aglom) and volume density (Vglom) of the renal cortex and medulla and volume density of glomeruli as well as the number of glomeruli per area (Naglom) were measured using a Zeiss eyepiece (Integrationsplatte II; Zeiss Co., Oberkochen, Germany) and the point counting method (Pglom = Aglom × Vglom) at a magnification of ×400. The Nglom was then corrected for tissue shrinkage (1.08²). Total cortex volume (Vcortex) was derived from kidney mass divided by specific weight of the kidney (1.04 g/cm³) times the volume density of the cortex. The number of glomeruli per cortex volume (Nglom) was derived from the Naglom and the Vcortex of glomeruli using the formula

\[N_{glom} = \frac{N_{aglom} \times \text{Volume density}}{\text{Vcortex}}\]

The mean glomerular volume (mVglom) was determined from Vcortex and number of glomeruli according to the formula

\[mV_{glom} = \frac{V_{cortex} \times N_{glom}}{N_{aglom}}\]

Analysis of Number and Volume of Glomerular Endothelial Cell, Fractional Glomerular Capillary Tuft Volume, Capillary Volume Density, Total Capillary Volume per Glomerulus, and Fractional Glomerular Mesangial Matrix Volume on Semithin Sections. On five semithin sections per animal, glomerular endothelial cell number and volume, as well as fractional capillary tuft volume, fractional mesangial matrix volume, and capillary volume density, were analyzed in at least 30 glomeruli per animal using the point-counting method and a 100-point eyepiece (Integrationsplatte II; Zeiss Co.) at a magnification of 1000 (oil immersion) as described previously (13).

Glomerular endothelial cell density was calculated from endothelial cell density per volume (NcV) and endothelial cells volume density (VcV) according to the equation

\[N_{cV} = \frac{k \times N_{aglom} \times V_{aglom}}{V_{vglom}}\]

where \(k = 1.1\) (size distribution coefficient) and \(\beta = 1.382\) (shape coefficient for spheres). The total number of glomerular endothelial cells was calculated from the Vcortex and the number of glomeruli per cortex volume (NcVglom)

\[N_{cV_{glom}} = N_{aglom} \times V_{cortex} \times N_{glom}\]

Stereologic Assessment of Glomerular Capillaries. Analysis of the glomerular capillaries was performed using the unbiased method according to Nyengaard et al. (9,10). For this purpose, five glomeruli per animal were randomly selected. In these glomeruli, the glomerulosclerosis score, according to El Nahas, was between 0 and 2. A topologic definition of capillary was used in this study: The generation of a new capillary loop corresponds to a change of one unit of Euler number of the network (9,10). The Euler number of glomerular capillary network (\(\chi_{\text{cap}}\)) is equal to 1 – connectivity. Connectivity is defined as the maximum number of cuts that can be made through the network without splitting the specimen into more parts (9,10). Therefore, in this study, the number of capillaries in glomeru-
The number of luminal convex figure \( P_{\text{glom}} \) and the number of points that hit the capillary were counted on each section. The number of glomerular tuft inside the minimal convex figure \( P_{\text{cap}} \). The point-counting technique was used. The estimation of Euler number was done by comparison of capillary lumina in two adjacent, parallel sections (Figure 1). Three different events were counted by the observer: Bridge (the division of capillary lumen into two or more capillary lumina), island (appearance of a capillary wall inside the lumen of a capillary profile), and hole (appearance of the isolated island of capillary lumen into two or more capillary lumina), island (appearance of the isolated island of capillary lumen). Examples of bridge and island are shown in Figure 1. The third topologic event—hole—is rare. It is presented schematically in Figure 2. Each capillary profile in the sample section was identified in the subsequent look-up section. The topologic events were counted. Then the sampled section was used as the look-up section for counting the topologic events from the opposite side of the dissector. The Euler number \( \Sigma \chi_{\text{cap}} \) was calculated as \( \Sigma \chi_{\text{cap}} = 1/2 \cdot \Sigma \text{no. islands} + \Sigma \text{no. holes} - \Sigma \text{no. bridges} \).

Two sets of light microscopes (Zeiss), video cameras (JVC TK 10-70E), and computer screens placed side by side (Nokia) were used. Five randomly selected glomeruli were studied per animal. Pairs of every sixth section of a sampled glomerulus were used as a dissector \( n = 10 \). The resulting images were projected simultaneously onto the computer screens with the use of video cameras. A system of coordinates divided the computer screen into 121 squares. Each square represented an area of 67 \( \mu \text{m}^2 \) of the examined cortical area \( a_{\text{P}} \).

A glomerulus was defined as the minimal convex figure enclosing the glomerular tuft. The point-counting technique was used. The number of points that hit the glomerular tuft inside the minimal convex figure \( P_{\text{glom}} \) and the number of points that hit the capillary lumen \( P_{\text{cap}} \) were counted on each section. The number of luminal profiles within the sampled section \( Q_{\text{lumen}} \) was also estimated. According to Nyengaard et al. (9) and Nyengaard and Marcussen (10), the numerical capillary density is equal to the Euler number density. The numerical capillary density (\( W_v \)) was calculated according to the formula \( W_v = \Sigma \chi_{\text{cap}}/2t \cdot \Sigma a_{\text{glom}} \), where \( t \) is the average section thickness (1 \( \mu \text{m} \)) and \( a_{\text{glom}} \) is the area of the glomerulus under investigation. The average number of capillaries per glomerulus \( W_{v_{\text{cap,glom}}} \) was calculated as \( W_{v_{\text{cap,glom}}} = \Sigma v_{\text{glom}} \times W_v \).

Figure 2. Schematic presentation of three topologic events used for calculation Euler number: Bridges, islands, and holes. Two consecutive semithin sections (look-up and sample) 1 \( \mu \text{m} \) thick are cut through capillaries (middle). The sections are shown as they are seen on the screen through the microscope (top and bottom).

**Figure 1.** The contribution to the Euler number \( \chi \) is shown using two consecutive, semithin stained sections. The observer looks for “topologic events” of capillary lumina: bridges, islands, and holes (see below). Each capillary profile in the sample section was identified in the following look-up section, and the topologic events were counted. Then a sample section was used as a look-up section for counting the topologic events in the opposite direction of the dissector. Four pairs of capillary lumina \( (A) \) on the look-up sections (to the left) connect with four single capillary lumina \( (C) \) seen on the sampled sections (to the right), forming so-called bridges. When the sequence is reversed, from right to left section, one pair of capillary lumina \( (A) \) connects with one capillary lumen \( (C) \). A capillary lumen on a sample section \( (F) \) is not seen on the look-up section \( (B) \). By definition, this is an island. The third topologic event, a hole (see Figure 2), was rare and is not seen on these sections.
Table 1. Animal dataa

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Weight at 8 Weeks (g)</th>
<th>Body Weight at 12 Weeks (g)</th>
<th>Left Kidney Weight (Intact or Remnant; g)</th>
<th>Plasma Creatinine (mg/dl)</th>
<th>Systolic BP (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 12 wk untreated (n = 6)</td>
<td>487 ± 15</td>
<td>562 ± 24</td>
<td>2.19 ± 0.12</td>
<td>0.50 ± 0.07</td>
<td>130 ± 11</td>
</tr>
<tr>
<td>SNX 8 wk (n = 6)</td>
<td>494 ± 34</td>
<td>—</td>
<td>2.98 ± 0.19f</td>
<td>0.89 ± 0.19f</td>
<td>154 ± 30</td>
</tr>
<tr>
<td>SNX 12 wk untreated (n = 6)</td>
<td>474 ± 37</td>
<td>543 ± 57</td>
<td>2.79 ± 0.55f</td>
<td>0.80 ± 0.10f</td>
<td>153 ± 18</td>
</tr>
<tr>
<td>SNX 12 wk delayed enalapril treatment (n = 6)</td>
<td>447 ± 27</td>
<td>483 ± 29g</td>
<td>2.35 ± 0.29f</td>
<td>0.91 ± 0.05f</td>
<td>121 ± 15bd</td>
</tr>
<tr>
<td>ANOVA</td>
<td>NS</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

a SNX, subtotally nephrectomized; NS, not significant.
b P < 0.05 versus SNX 8 wk.
c P < 0.01 versus SNX 8 wk.
d P < 0.05 versus SNX 12 wk untreated.
e P < 0.05 versus sham-operated rats.
f P < 0.01 versus sham-operated rats.

operated group (Table 1). Systolic BP values were significantly lower in SNX rats with delayed enalapril treatment.

Morphologic Analysis of the Kidney

Glomerulosclerosis Index. As shown in Table 2, the glomerulosclerosis index (GSI) was significantly higher in all SNX groups compared with sham-operated controls. At the end of the experiment, 12 wk after subtotal nephrectomy, the GSI was significantly lower in SNX rats with delayed enalapril treatment compared with untreated SNX rats. The GSI in SNX rats with delayed enalapril treatment was also significantly lower than in untreated SNX rats at 8 wk, suggesting not only prevention of progression but even partial reversal.

As shown in Figure 3, at 8 wk after subtotal nephrectomy, the glomerulosclerosis score in most of glomeruli was 1 (40.4%) and 2 (26.4%). Untreated SNX rats 12 wk after subtotal nephrectomy were characterized by a progressive increase of the frequency of sclerosed glomeruli. The percentage of glomeruli with scores of 2 (segmental hyalinosis/sclerosis involving <50% of the glomerular tuft) and 3 (segmental hyalinosis/sclerosis involving >50% of the glomerular tuft) tended to be higher in untreated rats at 12 wk after subtotal nephrectomy than in rats 8 wk after subtotal nephrectomy (36.6 versus 26.4% and 12.7 versus 8.3%, respectively). Comparison of the frequency of sclerotic glomeruli between treated and untreated SNX rats 12 wk after subtotal nephrectomy showed that the frequency of sclerosed glomeruli (scores 2 and 3) was significantly lower in SNX rats after delayed enalapril treatment compared with untreated SNX rats at 12 wk (9.8 versus 36.6% and 4.0 versus 12.7%, respectively). The frequency of sclerotic glomeruli with a score of 2 was significantly lower in SNX rats with delayed enalapril treatment at 12 wk compared with untreated SNX rats at 8 wk (9.8 versus 26.4%, respectively). The respective frequencies of glomeruli with score 3 were 4.0 versus 8.3%, respectively (P = 0.10). This observation is compatible with regression of glomerulosclerosis.

Table 2. Glomerular morphology

<table>
<thead>
<tr>
<th>Group</th>
<th>Glomerulosclerosis Index</th>
<th>Total No. of Glomeruli per Left Kidney</th>
<th>Mean Glomerular Volume (10³ μm³)</th>
<th>Fractional Glomerular Tuft Volume (%)</th>
<th>Mean Glomerular Tuft Volume (10⁶ μm³)</th>
<th>Fractional Mesangial Matrix Volume (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 12 wk untreated (n = 6)</td>
<td>0.08 ± 0.04</td>
<td>44,734 ± 8801</td>
<td>2.59 ± 0.44</td>
<td>80.3 ± 2.74</td>
<td>2.07 ± 0.35</td>
<td>3.51 ± 0.50</td>
</tr>
<tr>
<td>SNX 8 wk (n = 6)</td>
<td>1.25 ± 0.34e</td>
<td>17,482 ± 1440e</td>
<td>5.53 ± 0.88e</td>
<td>77.0 ± 5.05</td>
<td>4.33 ± 0.88e</td>
<td>9.93 ± 1.13e</td>
</tr>
<tr>
<td>SNX 12 wk untreated (n = 6)</td>
<td>1.61 ± 0.08e</td>
<td>15,038 ± 4544e</td>
<td>5.86 ± 0.89e</td>
<td>82.0 ± 4.18</td>
<td>4.78 ± 0.60e</td>
<td>11.0 ± 2.57e</td>
</tr>
<tr>
<td>SNX 12 wk delayed enalapril treatment (n = 6)</td>
<td>0.73 ± 0.20h,d,e</td>
<td>15,791 ± 2277e</td>
<td>4.60 ± 0.87e,c,e</td>
<td>76.6 ± 5.08</td>
<td>3.50 ± 0.57a,c,e</td>
<td>4.91 ± 1.21bd</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

a P < 0.05 versus SNX 8 wk.
b P < 0.01 versus SNX 8 wk.
c P < 0.05 versus SNX 12 wk untreated.
d P < 0.01 versus SNX 12 wk untreated.
e P < 0.01 versus sham-operated rats.
Glomerular Geometry. Subtotal nephrectomy lowered the total number of glomeruli per left kidney from an average of 44,734 to an average of 16,100. There was little variation among the three subtotal nephrectomy groups. Consistent reduction of nephron number was documented by the low degree of interindividual variation (Table 2).

The mean glomerular volume was significantly higher in all subtotal nephrectomy groups compared with sham-operated controls, indicating glomerular enlargement. Comparison of the groups shows that the mean glomerular volume was significantly less in SNX rats after delayed enalapril treatment compared with untreated SNX rats. Although the mean glomerular volume in untreated SNX rats at 12 wk tended to be higher than in untreated SNX rats at 8 wk (difference not statistically significant), it was significantly less in SNX rats with delayed enalapril treatment at 12 wk compared with untreated SNX rats at 8 wk, suggesting reduction of glomerular size.

Glomerular Capillary Tuft Volume and Mesangial Matrix Volume

As shown in Table 2, SNX rats did not differ significantly from sham-operated controls with respect to fractional glomerular capillary tuft volume. The mean tuft volume was significantly higher in all SNX groups than in sham-operated controls. The mean capillary tuft volume was significantly lower in SNX rats after delayed enalapril treatment compared with untreated SNX rats at 12 and at 8 wk (Table 2).

Fractional mesangial matrix volume was significantly higher in all SNX groups when compared with sham-operated controls. At the end of the experiment, 12 wk after subtotal nephrectomy, fractional mesangial matrix volume was significantly lower in SNX rats with delayed enalapril treatment compared with untreated SNX rats. It was also significantly lower in SNX rats with delayed enalapril treatment compared with untreated SNX rats at 8 wk, suggesting partial resolution of preexisting matrix accumulation (Table 2).

Capillary volume density was significantly lower in all SNX groups when compared with sham-operated controls. At the end of the experiment, i.e., 12 wk after subtotal nephrectomy, capillary volume density was significantly higher in SNX rats with delayed enalapril treatment compared with untreated SNX rats. It was also significantly higher in SNX rats with delayed enalapril treatment compared with untreated SNX rats at 8 wk (Table 3). Total capillary volume per glomerulus was significantly higher, in parallel with increased glomerular volume in all SNX groups when compared with sham-operated controls (Table 3).

Stereologic Assessment of Glomerular Capillaries. As shown in Table 3, the numerical capillary density (equivalent to Euler number density) was significantly less in SNX rats compared with sham-operated controls. The values did not differ in untreated SNX rats at 8 and 12 wk and SNX rats with delayed enalapril treatment at 12 wk (Table 3).

The number of capillaries per glomerulus was significantly higher in untreated SNX rats at 8 and 12 wk than in sham-operated controls (Table 3, Figure 4). The number of capillaries in glomeruli of SNX rats with delayed enalapril treatment was significantly lower compared with untreated SNX rats. It tended ($P = 0.08$) also to be lower than in SNX rats that were killed after 8 wk, suggesting reduction of the absolute number of capillaries by the enalapril treatment (Table 3).

The length density of glomerular capillaries was significantly lower in SNX rats compared with sham-operated controls (Table 3). Untreated SNX rats at 8 and 12 wk as well as SNX rats with delayed enalapril treatment at 12 wk did not differ with respect to length density of glomerular capillaries (Table 3).

The total length of capillaries per glomerulus was significantly higher in SNX rats compared with sham-operated controls (Table 3, Figure 5). The total length of capillaries per glomerulus in SNX rats with delayed enalapril treatment at 12 wk was significantly lower than in untreated SNX rats at 12 wk. Numerically, it tended also to be lower compared with SNX rats at 8 wk, suggesting a tendency of total length of capillaries per glomerulus to regress with enalapril treatment (Table 3, Figure 5).

The total capillary surface area per glomerulus was significantly higher in SNX rats than in sham-operated controls (Table 3). Total capillary surface area per glomerulus was significantly lower in SNX rats after delayed enalapril treatment compared with untreated SNX rats at 12 wk.

Stereologic Characteristic of Average Capillary. The length of the average capillary was significantly higher in SNX rats compared with sham-operated controls (Table 4). Untreated SNX rats at 8 and 12 wk and SNX rats with delayed enalapril treatment at 12 wk did not differ with respect to length of the average capillary (Table 4).

The groups did not differ with respect to the average capillary diameter (Table 4). The surface area of the average cap-
illary was significantly higher in SNX rats than in sham-operated controls (Table 4).

**Glomerular Endothelial Cell Number and Volume.** The mean number of endothelial cells per glomerulus was significantly higher in untreated SNX rats at 8 and 12 wk compared with sham-operated controls (Table 5). It was significantly lower in SNX rats after delayed enalapril treatment compared with untreated SNX rats at 12 wk or untreated SNX rats at 8 wk and did not differ significantly from sham-operated controls. The groups did not differ with respect to the endothelial cell number per individual capillary and the endothelial cell number/capillary length ratio (Table 5).

Mean endothelial cell volume and the endothelial cell volume/individual capillary length ratio tended to be higher in SNX groups compared with sham-operated controls. Untreated SNX rats at 8 and 12 wk and SNX rats with delayed enalapril treatment at 12 wk did not differ with respect to mean endothelial cell volume and the endothelial cell volume/individual capillary length ratio (Table 5).

**Qualitative Electron Microscopy.** Qualitative electron microscopic investigations of glomeruli confirmed activation and hypertrophy of glomerular endothelial cells in all SNX groups (Figure 6).

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**Table 3. Stereologic analysis of the glomerular capillary tuft**

<table>
<thead>
<tr>
<th>Group</th>
<th>Capillary Volume Density per Unit Volume of Tuft (µm³/µm³)</th>
<th>Total Capillary Volume per Glomerulus (10⁶ µm³)</th>
<th>Numerical Capillary Density per Unit Volume of Tuft (10⁻⁴ µm⁻³)</th>
<th>No. of Capillaries per Glomerulus</th>
<th>Length Density of Glomerular Capillaries (10⁻⁵ µm/µm³)</th>
<th>Total Length of Capillaries per Glomerulus (mm)</th>
<th>Total Capillary Surface Area per Glomerulus (10³ µm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 12 wk untreated (n = 6)</td>
<td>0.57 ± 0.03</td>
<td>0.56 ± 0.05</td>
<td>1.48 ± 0.27</td>
<td>149 ± 41</td>
<td>13.2 ± 1.5</td>
<td>13.0 ± 2.2</td>
<td>304 ± 38</td>
</tr>
<tr>
<td>SNX 8 wk (n = 6)</td>
<td>0.41 ± 0.03f</td>
<td>1.28 ± 0.37f</td>
<td>0.80 ± 0.08f</td>
<td>252 ± 81f</td>
<td>10.1 ± 0.7f</td>
<td>31.2 ± 8.3f</td>
<td>703 ± 191f</td>
</tr>
<tr>
<td>SNX 12 wk untreated (n = 6)</td>
<td>0.43 ± 0.08f</td>
<td>1.72 ± 0.34f</td>
<td>0.82 ± 0.27f</td>
<td>320 ± 66f</td>
<td>10.0 ± 1.6f</td>
<td>39.8 ± 7.8f</td>
<td>910 ± 163f</td>
</tr>
<tr>
<td>SNX 12 wk delayed enalapril treatment (n = 6)</td>
<td>0.50 ± 0.04b,c,e</td>
<td>1.36 ± 0.55f</td>
<td>0.71 ± 0.06f</td>
<td>197 ± 62d</td>
<td>8.6 ± 0.8f</td>
<td>24.1 ± 8.3d,e</td>
<td>605 ± 251c,f</td>
</tr>
</tbody>
</table>

ANOVA  

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**Figure 4.** Individual values and means of the capillary number per glomerulus in the four experimental groups (for statistics, see Table 3).

**Figure 5.** Individual values and means of the total capillary length per glomerulus in the four experimental groups (for statistics, see Table 3).

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\[ a \] Numerical capillary density is equal to Euler number density.

\[ b \] P < 0.01 versus SNX 8 wk.

\[ c \] P < 0.05 versus SNX 12 wk untreated.

\[ d \] P < 0.01 versus SNX 12 wk untreated.

\[ e \] P < 0.05 versus sham-operated rats.

\[ f \] P < 0.01 versus sham-operated rats.
Table 4. Stereologic characteristic of capillaries

<table>
<thead>
<tr>
<th>Group</th>
<th>Average Capillary Length (μm)</th>
<th>Average Capillary Diameter (μm)</th>
<th>Average Capillary Surface Area (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 12 wk untreated (n = 6)</td>
<td>95 ± 12</td>
<td>7.7 ± 2.1</td>
<td>2255 ± 375</td>
</tr>
<tr>
<td>SNX 8 wk (n = 6)</td>
<td>133 ± 9h</td>
<td>7.1 ± 0.3</td>
<td>2969 ± 256a</td>
</tr>
<tr>
<td>SNX 12 wk untreated (n = 6)</td>
<td>134 ± 22b</td>
<td>8.2 ± 2.0</td>
<td>3444 ± 796b</td>
</tr>
<tr>
<td>SNX 12 wk delayed enalapril treatment (n = 6)</td>
<td>124 ± 9b</td>
<td>7.8 ± 1.3</td>
<td>3036 ± 536a</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

a P < 0.05 versus sham-operated rats.
b P < 0.01 versus sham-operated rats.

Table 5. Glomerular endothelial cell number and volume

<table>
<thead>
<tr>
<th>Group</th>
<th>Endothelial Cell Number per Glomerulus</th>
<th>Endothelial Cell Number per Average Capillary</th>
<th>Endothelial Cell Number/Capillary Length Ratio (10⁻⁵ μm⁻¹)</th>
<th>Mean Endothelial Cell Volume (μm³)</th>
<th>Endothelial Cell Volume/Average Capillary Length Ratio (μm²/μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham 12 wk untreated (n = 6)</td>
<td>255 ± 45</td>
<td>1.83 ± 0.63</td>
<td>2.02 ± 0.58</td>
<td>124 ± 19</td>
<td>1.35 ± 0.35</td>
</tr>
<tr>
<td>SNX 8 wk (n = 6)</td>
<td>554 ± 169d</td>
<td>2.51 ± 1.27</td>
<td>1.98 ± 1.00</td>
<td>191 ± 69</td>
<td>1.46 ± 0.59</td>
</tr>
<tr>
<td>SNX 12 wk untreated (n = 6)</td>
<td>535 ± 121d</td>
<td>1.74 ± 0.68</td>
<td>1.35 ± 0.39</td>
<td>217 ± 28d</td>
<td>1.66 ± 0.41</td>
</tr>
<tr>
<td>SNX 12 wk delayed enalapril treatment (n = 6)</td>
<td>330 ± 75ah</td>
<td>1.80 ± 0.74</td>
<td>1.51 ± 0.73</td>
<td>236 ± 37d</td>
<td>1.91 ± 0.26c</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.001</td>
<td>NS</td>
<td>NS</td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>
a P < 0.01 versus SNX 8 wk.
b P < 0.01 versus SNX 12 wk untreated.
c P < 0.05 versus sham-operated rats.
d P < 0.01 versus sham-operated rats.

Discussion

The present study evaluated capillary remodeling during partial reversal of glomerular lesions after subtotal nephrectomy. This was assessed by comparing untreated SNX and SNX rats with established glomerulosclerotic lesions after delayed administration of high doses of an angiotensin-converting enzyme (ACE) inhibitor. That the frequency of glomeruli with advanced lesions was the lowest in the group of treated animals is consistent with the concept of reversal of glomerular injury. Although we did not formally exclude the possibility that histology improved because of dropout of the more advanced lesions, this is not extremely likely, however, given the short time interval of 4 wk. Moreover, we did not see sclerosed glomeruli undergoing the process of resorption. The extremely high doses of ACE inhibitor are presumably necessary because the local intrarenal renin-angiotensin system(s) is not affected even by maximally BP lowering doses of ACE inhibitors, as documented by Nishiyama et al. (16).

The salient feature of the study was the amazingly rapid, parallel, and coordinated reduction of mean glomerular volume, tuft volume, and capillary number with a tendency for reduced average capillary length. The proportion of the tuft volume comprised by mesangial matrix decreased, but the numerical density of the capillaries per unit volume of tuft remained unchanged. The reduction of the total tuft volume necessitates remodeling of the microvasculature, and this is achieved by reduction of the total capillary number per glomerulus (and in parallel, the total number of endothelial cells) with modest, if any, change in length and radius of individual capillaries.

The correct interpretation of the data requires discussion of some critical methodologic points. The numerical capillary density, i.e., the Euler number density (9,10), did not change. As proposed by Burri and Tarek (17), formation of slender intravascular tissue pillars (intussusceptive growth) leads to new capillary formation in the lung and as recently reported (18) also in the kidney. This process will cause a change in the Euler number.

We studied a limited number of representative glomeruli per animal following the procedure of previous authors (19–21). A potential methodologic problem arises, however, when there are interglomerular or intraglomerular inhomogeneities of the lesions. Remuzzi et al. (22) used three-dimensional morphometric analysis of glomeruli and exhaustive sampling of lesions in nearly all glomeruli. They showed both in a nonablation rat model of glomerulosclerosis and in humans with extensive renal mass reduction (23) that focal areas of sclerosis occupied only a small proportion of glomerular tuft volume. With the methods used in the current study, we are not able to
characterize separately capillary microarchitecture in glomerular areas affected or not by sclerosis.

If one measures only the relative frequency of capillaries, i.e., the numerical capillary density and the length density, then one might be misled to believe that capillary rarefaction had occurred after subtotal nephrectomy (see Table 3). The advantage of the powerful stereologic analysis is that measurements in three-dimensional space reveal that, pari passu with the increase in glomerular volume, total capillary length and capillary number per glomerulus had actually increased. The geometry of the individual capillary is only slightly altered with a moderate increase in average capillary length, no change in intraindividual variance (data not shown), and no change in average capillary diameter.

A brief discussion concerning the topologic definition of a capillary that had been adopted for this analysis is helpful. According to Nyengaard et al. (9,10), “When a new capillary is generated, a new loop in the capillary network is created corresponding to a change of exactly 1 unit, the Euler number of the network.” This means that a new capillary starts as a budding out of endothelial cells through the basement membrane of an existing capillary wall or, alternatively, by intussusception (17,18). We emphasize that this definition of Nyengaard et al. (9,10) differs from what has been used in some studies on glomerular capillaries in which a capillary was defined as the segment between two nodes in the network (24–26).

There have recently been several papers on changes in the glomerular capillary network occurring during initial development of glomerular lesions and the topologic details of this process (19–21). Previous authors emphasized the similarities and potentially analogous mechanistic pathways when microvascular repair and glomerular development are compared (8,27). An early proliferative response of glomerular endothelial cells during the development of glomerular lesions has also been documented (28). This is not sustained, however, and in the long run, even loss of glomerular endothelial cells is observed (28,29).

Only limited information is available on the fine morphologic details during regression of glomerular lesions. One exception is a study (8) in the anti–Thy-1 nephritis model that does not allow, however, direct comparison with reversal of glomerulosclerosis in the noninflammatory renal ablation model.

The results of the present study document that reversal of glomerular lesions is not a haphazard process but involves highly coordinated glomerular remodeling with parallel and quantitatively proportional reductions of glomerular structures, e.g., parallel reduction of the volumes of Bowman’s space and of the tuft, reduction of endothelial cell number, and removal of capillaries without major shortening of capillaries or changes of capillary diameter. Because of the regression of glomerulosclerotic lesions, one would have anticipated a decrease of the number of capillaries per glomerulus and of total capillary length in enalapril-treated SNX rats (comparison between untreated SNX rats at 8 wk and SNX rats treated with enalapril between 8 and 12 wk). Only a nonsignificant trend for reduced numbers of capillaries per glomerulus and of total capillary length was observed after enalapril treatment. Our finding of persistently increased endothelial cell volume may reflect persistently altered hemodynamics and shear stress or alternatively may be due to increased metabolic activity. Overall, what happens during reversal is reminiscent of the converse process when glomeruli enlarge: Nyengaard and Rasch (20) found in a diabetes model that the number of capillaries increased, whereas the capillary length was changed little if at all. This seems to be a general pattern because the same pattern was found in lithium nephropathy (19). After adult nephrectomy of the rat, capillary growth involves new capillary formation instead of simple thickening of existing capillaries (21). However, the data of the uninephrectomy model (21) cannot be compared directly with the results of the more aggressive renal ablation model used in the present study.

The results of the current study clearly showed that during regression of glomerulosclerotic lesions after subtotal nephrectomy, the average capillary geometry is largely stable (i.e., length, surface area, and diameter). Moreover, the cellular characteristics of the average capillary did not change (i.e., endothelial cell number per average capillary, endothelial cell number/capillary length, and endothelial cell volume/average capillary length ratio). This finding suggests that highly coordinated glomerular remodeling occurs on the level of the individual capillary as well.

The estimation of endothelial cell number and volume was done with a modified Weibel-Gomes method on the basis of the assessment of cell density. For this approach, it was assumed that the shape of endothelial cells in all groups was the same. We do not have data, specifically not systematic ultra-thin and electron microscope sections, to verify this assumption, however. This source of error is not extremely likely,
because we did not find major changes in endothelial cell shape by inspection, but we acknowledge that in principle the endothelial cell shape might be changed in the presence of glomerulosclerosis.

The signals that are almost certainly operative in orchestrating these processes are unknown. Unfortunately, the necessary fixation of tissues precluded immunohistologic and molecular analysis.

This study shows coordinate remodeling of the entire glomerulus during regression of glomerular lesions with proportionate reduction of glomerular and tuft volume as well as of capillary number but without major change of individual capillary length and diameter.

Appendix

The volume fraction of capillary lumina \( V_{v(cap/glom)} \) was calculated as \( V_{v(cap/glom)} = \frac{\Delta P(cap)}{\Delta P(glom)} \).

The length density of glomerular capillaries \( L_{v(cap/glom)} \) was calculated according to the formula \( L_{v(cap/glom)} = \frac{L_{cap} a(p)}{P_{cap} P_{glom}} \).

The average capillary cross section area \( a(cap) \) was calculated as \( a(cap) = \frac{V_{v(cap/glom)} L_{v(cap/glom)}}{\Delta P(cap)} \).

The average capillary surface area \( s(cap) \) was calculated as \( s(cap) = \frac{V_{v(cap/glom)} L_{v(cap/glom)}}{\Delta P(cap)} \).

The average capillary diameter \( d(cap) \) was calculated as \( d(cap) = \frac{S(cap) w(cap)}{\pi} \).

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